REMARKS

Claims 1, 17, and 19 have been amended, and new claims 20 and 21 have been added. In particular, claims 1, 17, and 19 have been amended to recite that the plant material is protonema tissue "transformed with a construct encoding a signal peptide operably linked to a protein" as supported on page 7, lines 11-15, of the specification as originally filed, and as is acknowledged to be supported by the Examiner's admission on p. 2 of the Office Action dated March 14, 2006. Applicants note that, in reciting "a construct," "a signal peptide," and "a protein," Applicants intend to encompass both the singular and the plural in accordance with the usual practices of claim construction. Claims 1, 17, and 19 are also amended to recite "obtaining secreted heterologous proteinaceous substances," as supported by, e.g., the specification at page 5, lines 26-27.

New dependent claims 20 and 21 pertain to bryophyte species having well known transformation protocols at the time the invention was made.

No new matter has been added to the present application by the amendment.

The Invention

The present invention pertains generally, as recited in amended claim 1, to a method for producing heterologous substances in plant material, comprising the steps of culturing plant material in a culture medium, wherein the plant material is protonema tissue, **transformed with a construct encoding a signal peptide operably linked to a protein**, that produces heterologous proteinaceous substances; and obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. In short, the invention relates to solving the problem of **obtaining secreted heterologous proteinaceous substances from intact protonema tissue, which is made up of cells having cell walls**.

In accordance with another method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in transformed protonema tissue from *Physcomitrella* is provided having the steps recited in claim 17.

In accordance with yet another method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in transformed protonema tissue from *Physcomitrella patens* is provided having the steps recited in claim 19.

Various other embodiments in accordance with the present invention are recited in the dependent claims. All of the embodiments in accordance with the present invention provide a method for producing heterologous proteinaceous substances in plant material, whether protonema moss tissue or protonema liverwort tissue, wherein the proteinaceous substances produced by the transformed protonema tissue are advantageously obtained without disrupting producing tissues or cells.

Persons skilled in the art would recognize that the present invention advantageously utilizes protonema tissue, wherein "protonema" is defined in the art as "the usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts comparable to the prothallium in ferns" (See Webster's New Collegiate Dictionary, 1977, p. 927) (of record).

One aspect of the novelty and unobviousness of the present invention over the prior art relates to the step of "obtaining **secreted** heterologous proteinaceous substances...without disrupting producing tissues or cells" because persons skilled in the art would not have predicted that proteinaceous substances could be obtained from transformed protonema tissue, i.e., mature and phototropic plant tissue made up of cells having cell walls, without disrupting the producing tissues or cells.

The Rejections

Claims 1-6 and 17 stand rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. The Examiner admits that the application is enabled for a method for the production of *Physcomitrella patens* by transformation with constructs that encode signal peptides operably linked to the proteins. Office Action dated March 14, 2006, at 2, lines 9-12. Using this admission, applicants have amended all claims to require protonema "transformed with a construct encoding a signal peptide operably linked to a protein." Thus, the only remaining issues with respect to enablement are whether the application enables protonema tissue within the scope of the various claims beyond simply *Physcomitrella patens*. For the reasons which follow, the voluminous, uncontested evidence of record, in addition to the Examiner's admissions, shows that the claims as presently presented are enabled.

Claims 1-5, 17 and 18 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Houba-Hérin et al. (Nichole Houba-Hérin et al., *Cytokinin oxidase for Zea mays: purification,*

cDNA cloning and expression in moss protoplasts. 17 The Plant Journal 615, 615-626 (1999)) (hereinafter, the "Houba-Hérin article") in view of Reutter et al. (K. Reutter and R. Reski, *Production of a heterologous protein in bioreactor cultures of fully differentiated moss plants*. 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996)) (hereinafter, the "Reutter article").

Claim 6 stands rejected under 35 U.S.C. § 103(a) as unpatentable over the Houba-Hérin article in view of the Reutter article, and further in view of Nasu et al. (M. Nasu et al., *Efficient transformation of Marchantia polymorpha that is haploid and has very small genome DNA*. 84 J. Ferm. Bioengin. 519, 519-523 (1997)) (hereinafter, the "Nasu article").

Claims 1-5, 17 and 18 also stand rejected under 35 U.S.C. § 103(a) as unpatentable over the Reutter article in view of Raskin (U.S. Patent 6,096,546, hereinafter, the "Raskin Patent").

In view of the present amendment, Applicants respectfully traverse the rejection and request reconsideration for the following reasons.

APPLICANTS' ARGUMENTS

I. THE REJECTION UNDER 35 U.S.C. § 112 IS UNTENABLE

A. The Rejection is Not Appropriately Applied to the Claims and Is Unsupported by Facts

Claims 1-6, 17 and 19-21 comply with 35 U.S.C. § 112, first paragraph. Applicants assert that the Examiner has not established a *prima facie* case of lack of enablement under 35 U.S.C. § 112, first paragraph for the numerous reasons recited below, and that even if a prima *facie* were made out, Applicants have submitted more than sufficient evidence to overcome it.

The Examiner also admits that the application is enabled for a method for the production of *Physcomitrella patens* by transformation with constructs that encode signal peptides operably linked to the proteins. Office Action dated March 14, 2006, at 2, lines 9-12. As discussed above, applicants have amended all claims to require protonema "transformed with a construct encoding a signal peptide operably linked to a protein." Thus, the only remaining issues with respect to enablement are whether the application enables protonema tissue within the scope of the various claims beyond simply *Physcomitrella patens*.

The Examiner admits that the present application is enabling for a method for the production of secreted proteins in *Physcomitrella patens* (Office Action dated March 14, 2006, at 2, lines 9-12), which is a bryophyte. For this reason, the Examiner's enablement rejection cannot apply to claim 19, which pertains to such a method. In particular, claim 19 recites a "method for the production of heterologous proteinaceous substances in plant material, comprising the steps of culturing plant material in a culture medium, wherein the plant material is protonema tissue, transformed with a construct encoding a signal peptide operably linked to a protein, that produces heterologous proteinaceous substances, wherein the protonema tissue is *Physcomitrella patens*...." Therefore, for the reasons of record, claim 19 complies with the enablement requirement of 35 U.S.C. § 112.

However, the Examiner asserts that no other bryophytes, not even any of the other four members of the *Physcomitrella* species, are reasonably enabled by the present disclosure on the grounds that application of the heterologous gene in accordance with the present invention to any other bryophyte would require undue trial and error experimentation (Office Action dated March 14, 2006, at page 2, lines 12-13 and at page 3, lines 14-17). The Examiner also asserts that the specification fails to provide guidance for a method for production of secreted proteins from

moss or liverwort tissue when the protein is not produced with a signal peptide (Office Action dated March 14, 2006, at 3, lines 8-9). **None of these assertions are supported by any factual evidence, apart from a now-irrelevant reliance on Baur et al.** (2005, J. Biotechnol. 119:332-342) (hereinafter the "Baur article"), addressed below). The Examiner's conclusory statements of non-enablement (e.g., "methods within the full scope of the claims would be beyond one of ordinary skill in the art") (Office Action dated March 14, 2006, page 6) are unsupported by any facts or evidence in the record.

Moreover, the Examiner's second assertion is moot in view of the present claim amendment. The claims, as amended, are directed to a method for the production of heterologous proteinaceous substances, the method comprising culturing protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein, and obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells.

Furthermore, in accordance with the Manual of Patent Examining Procedure (MPEP) § 904.01, the Examiner should "be fully aware of what the claims do *not* call for, as well as what they do require" (emphasis in the original). In the present case, the step of transforming mosses and liverworts and growing them to the protonema stage is not an element of the claimed invention. Therefore, the Examiner should refrain from reading this step from the specification into the claims. See Texas Digital Systems Inc. v. Telegenix Inc., 64 U.S.P.Q.2d 1812, 1819 (Fed. 2002). Thus, the rejection additionally fails because it does not recognize that the present invention pertains to a method comprising the steps of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. Instead, the Examiner appears to be interpreting the claims to include steps (a) and (b), and the step of "transforming" protoplast cells (Office Action, dated March 14, 2006, at 4, lines 1-3; Office Action dated June 7, 2005, at 4, lines 16-20). However, the transformation of bryophyte protoplasts is well known in the art for many species of bryophytes, and is not an element of the claim. The claims are not directed at genetically transforming tissue, but at culture tissue that is already transformed.

Regarding the Baur article, although the Examiner has cited it in support of failure when signal peptide is lacking (Office Action dated March 14, 2006, page 3), this argument is moot in view of the amendments to the claims, which now recite the signal peptide limitation. The Baur article, now irrelevant, was the only evidence presented in support of non-enablement.

In summary, the rejections under § 112 are either moot in view of the present amendment, or are unsupported by any facts. Applicants point out that, as a matter of law, it is not necessary to enable every species in a genus encompassed by the claims in order to meet the enablement requirement for the genus: the enablement analysis must be made on a case-by-case basis. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). Enabling "the full scope of the claims" does not require complete working examples for every conceivable species. Id. Below Applicants set forth the multitudinous evidence of record showing that the claims are enabled.

B. All of the Evidence of Record Supports a Finding of Enablement

Applicants do not rely on argument alone, but on facts. Attached herewith is a Declaration under 37 C.F.R. § 1.132 by Ralph Reski (hereinafter, the "Reski Declaration"), co-inventor of the above-captioned application and an internationally recognized leading researcher in the field of bryophyte biology. Dr. Reski agrees with the previous testimony by Dr. Gilbert Gorr, another co-inventor of the above-captioned application and another internationally recognized researcher in the field of bryophyte biology, established in the Declaration under 37 C.F.R. § 1.132 by Gilbert Gorr (hereinafter, the "Gorr Declaration"). According to the testimony of Dr. Reski and Dr. Gorr, a person skilled in the art would expect other members of the *Physcomitrella* species to behave in the same way under the same cell culture conditions as *Physcomitrella patens* (Reski Declaration, ¶¶ 5 and 6; Gorr Declaration, ¶¶ 5 and 6). Furthermore, the experimental evidence provided by the Gorr Declaration shows that even more distant bryophyte relatives, such as *Funaria hygrometrica* (a moss) and *Marchantia polymorpha* (a liverwort), can be successfully transformed by routine experimentation using the transformation protocol described in the instant application (Gorr Declaration, ¶¶ 15-18). Dr. Reski's testimony and Dr. Gorr's testimony also establish that cultivation and transformation of bryophytes, including both mosses and liverworts, is mature and well developed, and that a person of ordinary skill in the art would have known, at the time the invention was made, how to

transform and culture other *Physcomitrella* species, *Ceratodon* species, *Marchantia* species, and many other species of bryophytes as well because "bryophytes are simple, primitive plants that are expected to behave biologically in a relatively uniform manner" (Reski Declaration, ¶49; Gorr Declaration, ¶ 30).

The Examiner has dismissed the declaratory evidence as unpersuasive, but, as stated elsewhere herein, has done so without presenting evidence of any kind in rebuttal. The Examiner maintains that Dr. Gorr, for example is one of extraordinary skill in the art. This criticism is irrelevant, since his testimony relates entirely to what one of ordinary skill in the art would be able to do, and it is undisputed that he is competent to opine on that subject.

The Examiner admits that (i) "Zeidler et al. disclose transformation of the moss *Ceratodon purpureus* (pg 643-647)" (October 4th Office Action, p. 6, lines 17-19) and also that (ii) "Nasu et al. teach transformation of *Marchantia polymorpha* (pg 520, left column, paragraphs 1-2)," which is a liverwort (March 14th Office Action, at 13, lines 5-6; October 4th Office Action, at 6, lines 1-6). To be applied as prior art, such references must be enabling. In re Donohue, 226 U.S.P.Q. 619, 621 (Fed. Cir. 1985).

The Examiner's arguments are contradictory. Specifically, the Examiner admits that the present invention is enabled for *Physcomitrella patens* and also points out, based on the prior art, that a person of ordinary skill in the art would know how to genetically transform additional moss species (i.e., *Ceratodon purpureus*) and additional liverwort species (i.e., *Marchantia polymorpha*). The Examiner also argues that it would be obvious to do so on the grounds that "substitution of one bryophyte for another is an obvious optimization of design parameters" (March 14th Office Action, at 13, lines 8-12; October 4th Office Action, at 6, lines 10-12; and Office Action dated June 7, 2005, at 7, lines 18-19). The Examiner has in fact correctly established, based on citation of the prior art, that the transformation of mosses and liverworts is well known and predictable.

In view of the facts admitted by the Examiner, and the additional facts established by the Reski Declaration and the Gorr Declaration, Applicants traverse the Examiner's grounds for the present lack of enablement rejection under 35 U.S.C. § 112 standing against the instant claims because the Examiner has produced no evidence grounded in the prior art in support of this rejection, and has evinced no sustainable reason for the rejection. It is the Examiner's burden to

set forth a reasonable explanation as to why the scope of protection sought is not enabled by the description of the invention. In re Wright, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993). Specifically, the Examiner has provided **no** evidence (i.e., article, textbook, technical disclosure, etc.) to show, or even suggest, that by enabling the invention for *Physcomitrella patens* (as has been admitted by the Examiner) the Applicants have not enabled the invention for protonema tissue within the scope of the claims.

On the contrary, the evidence adduced by the Examiner based on the prior art of record, such as Nasu article, actually supports the conclusion that the present invention is enabled for both mosses and liverworts. This is not a case where the Examiner has provided some evidence, such as a published article, to establish the unpredictability of the target class of organisms. See In re Vaeck, 20 U.S.P.Q2d 1438, 1445 (Fed. Cir. 1991) (Claimed subject matter related to transforming cyanobacteria, a diverse and relatively poorly understood group of microorganisms); and In re Wright, 27 U.S.P.Q.2d 1510, 1513-4 (Fed. Cir. 1993) (Examiner produced an article teaching that the physiologic activity of RNA viruses was sufficiently unpredictable to establish that all living organisms could be immunized against infection by any pathogenic RNA virus by inoculation with a live virus containing the antigenic code, but not the pathogenic code, where applicant provided only one working example). The only evidence supplied by the Examiner actually weighs in favor of the Applicant's argument.

This is a case where the Examiner has provided absolutely no evidence to show, or even suggest, that bryophytes are poorly understood and/or unpredictable organisms. On the other hand, Applicants have adduced the testimony of two experts in the field in support of the contention that bryophytes are well-studied, predictable organisms. In the present case, the Examiner has simply failed to establish a *prima facie* case of lack of enablement under 35 U.S.C. § 112, as a matter of law, because the Examiner has provided no reasonable basis for the rejection.

C. The Elements of Enablement

The statutory enablement requirement of 35 U.S.C. § 112, first paragraph, is a question of law based on underlying facts as to whether a specification teaches those of ordinary skill in the art how to make and use the full scope of the claimed invention without undue experimentation.

In re Wands, 858 F.2d 731, 735 (Fed. Cir. 1988); In re Wright, 27 U.S.P.Q.2d at 1513. The initial burden rests on the Examiner to provide sufficient reasons for doubting assertions in the specification as to the scope of enablement. In re Wright, 27 U.S.P.Q.2d at 1513. In particular, the number of representative species required to support a particular genus is a matter of fact. Johns Hopkins Univ. v. Cellpro, Inc., 152 F.3d 1342, 1359 (Fed. Cir. 1998).

In the present case, the Examiner admits the specification is enabling to those of ordinary skill in the art for the bryophyte *Physcomitrella patens* producing heterologous protein associated with transit peptide and secreting the protein into the culture medium, and the Examiner herself has produced evidence that a person skilled in the art would know how to transform *Ceratodon purpureus* and *Marchantia polymorpha* without undue experimentation. The wealth of information uncovered by the Examiner teaches that the field of bryophyte transformation is mature, well developed and predictable. This same conclusion follows from both Dr. Reski's and Dr. Gorr's review of relevant scientific literature and from the results of additional experiments demonstrating the ease of transforming other bryophyte species (Reski Declaration, ¶49; Gorr Declaration, ¶ 30).

Despite overwhelming evidence to the contrary, the Examiner asserts that the heterologous gene inserted into a single species or subspecies of bryophyte would not enable an ordinary person skilled in the art to practice the claimed invention without undue experimentation in any other bryophytes, not even closely related mosses such as *Funaria*, *Sphagnum*, *Ceratodon* and other *Physcomitrella* subspecies (March 14th Office Action, at 3, lines 14-17; October 4th Office Action, at 3, lines 3-13; Office Action of June 7, 2005, at 2, line 15, to at 3, line 12). The Examiner's argument necessarily fails in view of the Examiner's admission that it is well known in the art to transform mosses, such as *Ceratodon*, and liverworts, such as *Marchantia*. The argument especially fails in view of the additional experimental evidence submitted in the Gorr Declaration demonstrating how easy it is to transform even relatively distant species, such as *Funaria hygrometrica*, and liverworts, such as *Marchantia polymorpha* (Gorr Declaration, ¶¶ 14-18). In fact, all of the evidence of record supports the notion that the claimed genus is enabled.

As a matter of law, it is not necessary to enable every species in a genus encompassed by the claims in order to meet the enablement requirement for the genus: the enablement analysis

must be made on a case-by-case basis. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). A rejection based solely on the size of the genus claimed is insufficient. Id. Thus, Examiner's assertion that "the working example does not teach species within the full scope of the claims" (Office Action of March 14, 2006, page 7, lines 6-7) is insufficient to support the rejections.

Enablement is not precluded by the necessity for some experimentation; rather, the question is whether the amount of experimentation is "undue experimentation" as determined upon the weighing of many factors. In re Wands, 8 U.S.P.Q.2d 1400, 1404. Factors to consider when determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. Id.

The Examiner contends that the specification does not enable the claimed genus (in the patent law sense) because the amount of experimentation required to transform other mosses and liverworts would amount to "undue trial and error experimentation." (Office Action, dated October 4, 2004, page 3, lines 10-13). Again, this assertion has no support in the record. Applicants disagree with the Examiner's unsupported conclusions for the following reasons.

D. Analysis of the *Wands* Factors Shows the Claims Are Enabled

Claim 1 recites (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. An analysis of the Wands Factors for methods for the production of heterologous proteinaceous substances in plant material in accordance with claims 1-6, 17 and 19-21 is as follows.

1. The Specification Provides Abundant Direction and Guidance

First, the present specification gives ample guidance regarding how to practice and use the claimed invention as recited in claims 1-6, 17 and 19-21. Specifically, the specification supplies abundant guidance regarding how to use intact plants to obtain heterologous proteinaceous substances directly from the culture medium without disrupting producing tissues

or cells (See specification, page 9, line 5, to page 27, line 26). In other words, the present specification provides about 18 pages of detailed instruction.

Also, well-known and commercially available starting materials for practicing the methods are known in the prior art (see page 7, lines 1-8, and page 9, lines 6-24), with materials for a detailed example described on page 11, line 29, to page 26, line 18, of the present specification. In particular, the specification describes multiple species of mosses and liverworts that are well-known, characterized, and previously studied and that are suitable for practicing the present invention, including *Physcomitrella*, *Funaria*, and *Ceratodon* species (See specification, page 9, lines 6-24, and R. Reski, *Development, genetics and molecular biology of mosses.* 111 Bot. Acta 1, 1-15 (1998) (of record), hereinafter the "Reski article") as well as *Sphagnum* species (See specification, page 9, lines 6-18, and H. Rudolph and S. Rasmussen, *Studies on secondary metabolism of Sphagnum cultivated bioreactors.* 3 Crypt. Bot. 67, 67-73 (1992) (of record), hereinafter the "Rasmussen article").

Furthermore, the specification gives detailed direction and guidance regarding the genetic transformation of the species *Physcomitrella patens* so that its protonema tissue produces vascular endothelial growth factor VEGF (See specification as originally filed, page 11, line 29, to page 18, line 35). The specification explains that genetic transformation systems for *Physcomitrella patens* have been previously developed and described for enzyme production (See specification, page 9, line 26, to page 10, line 2, and K. Reutter and R. Reski, *Production of heterologous protein in bioreactor cultures of fully differentiated moss plants.* 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996) (of record)). The detailed amount of direction and guidance provided by the specification of the above-captioned application has lead Dr. Reski to conclude that a person of ordinary skill in the art would be able to carry out the invention of independent claims 1, 17 and 19 without undue experimentation (Reski Declaration, ¶¶ 22-25 and 49).

In view of the above facts demonstrating the application of gene transformation techniques to multiple bryophyte species developing protonema tissue, it is evident that a person of ordinary skill in the art would only have to apply routine experimentation to adapt the method described by the specific example provided by the instant specification, wherein the plant protonema tissue is *Physcomitrella patens*, to other protonema tissue whether belonging to

mosses or liverworts. This conclusion, based on the direction and guidance provided by the disclosure of the above-captioned application, is bolstered by the fact that when such additional experiments are carried out by those of ordinary skill in the art, successful transformation of other species of mosses and liverworts can be achieved without undue experimentation (See Gorr Declaration, ¶¶ 18 and 30).

Thus, as Dr. Reski has concluded, the first Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

2. Working Example Are Present

Second, the present specification provides a specific enabling example in the disclosure to teach how to perform and use the "method for the production of heterologous proteinaceous substances in plant material...wherein the plant material is protonema tissue" as described on page 11, line 29, to page 18, line 35 of the specification as originally filed. Protonema tissue is a well-known plant tissue type, which manifests numerous common biological characteristics and functions whether originating from moss or liverwort species. See Reski Declaration, ¶¶ 26-27, and Gorr Declaration, ¶¶ 5-22.

Thus, as Dr. Reski has concluded, the second Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

3. The Nature of the Invention and State of the Art Support Enablement

Third, the present invention is directed to a method of producing heterologous proteinaceous substances using genetically transformed protonema tissue. Thus, the method in accordance with this embodiment of the present invention employs simple plant organisms that have a well-characterized plant physiology and predictable developmental cycle (See R. Reski, *Development, genetics and molecular biology of mosses*. 111 Bot. Acta 1, 3, 6 and 11 (1998); and the Nasu article, at 519, first column, lines 1-24). In other words, the biological plant organisms producing protonema tissue, whether of the moss phylum (e.g., *Physcomitrella*, *Funaria*, *Sphagnum* and *Ceratodon*) or the liverwort phylum (e.g., *Marchantia* and *Sphaerocarpos*), are relatively simple, predictable organisms as supported by Dr. Reski's testimony (Reski Declaration, ¶ 28). The State of the Art is mature as evident from such prior art

references as the Houba-Hérin article, the Reutter article, the Zeidler article, the Nasu article, the Rasmussen article, and the review article by H. Mühlbach, *Use of plant cell cultures in biotechnology*. 4 Biotechnology Annual Review 113, 158-161 (1998) (specifically, page 158, line 32, to page 161, line 6 (of record)) (hereinafter, the Mühlbach article). In fact, the Mühlbach article states that

> [t]hese studies document the advanced stage that is currently achieved in the genetic transformation of *P. patens*, which can be certainly extended to other genes and also to other bryophytes with potential use in biotechnology. Evidence along this line comes from studies on the expression of the human vascular endothelial growth factor (VEGF) protein in bioreactor cultures of *P. patens*....In general, these promising approaches clearly demonstrate the feasibility of bioreactor cultures of transgenic mosses for the production of heterologous compounds.

H. Mühlbach, at page 160, line 38, to page 161, line 6, (emphasis added).

In addition, the primary references recited against the claims of the present application, such as the Houba-Hérin article, the Reutter article, the Zeidler article and the Nasu article, were published about 5 or more years ago, which further suggests the mature nature of the relevant art. Lastly, following a review of relevant scientific literature, both Dr. Reski and Dr. Gorr testify that the state of the art regarding the cultivation and transformation of bryophytes, including both mosses and liverworts, is mature and well developed (Reski Declaration, ¶ 28; Gorr Declaration, ¶ 30).

Thus, as Dr. Reski has concluded, the third and fourth Wands factors weigh in favor of enablement (Reski Declaration, ¶49).

4. The Relative Skill of Those in the Art is Very High

Fourth, as is generally known, persons of ordinary skill in the art of transforming plant cells to express selected proteins, such as human VEGF, are highly trained professionals with advanced degrees in cellular and molecular biology who are involved in research and technological advancement of the field. The relative skill level of those in the art is notably high (See, e.g., the Curriculum Vitae of Dr. Gilbert Gorr, of record), as Dr. Reski testifies (Reski Declaration, ¶ 32).

Thus, as Dr. Reski has concluded, the fifth Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

5. The Art is Predictable

Fifth, Applicants point to the Zeidler article and the Nasu article cited by the Examiner as showing that the transformation of other protonema forming species, such as *Ceratodon* and *Marchantia*, are known. The predictability of the art, pertaining to the transformation of plant species that ultimately produce protonema tissue, is highly predictable. This fact is additionally supported by the Gorr Declaration, ¶¶ 11-18, and the Reski Declaration, ¶¶ 34-35. The facts attested to therein show that one of ordinary skill in the art can easily apply the present teachings to the full scope of the claims with only routine experimentation, because the art is predictable. The Examiner has produced zero evidence to the contrary.

Moreover, the claims do not even require such transformation, but merely cultivation and obtaining proteins. These arts are quite predictable.

Thus, as Dr. Reski has concluded, the sixth Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

6. Only Routine Experimentation Is Necessary

Sixth, the present specification generally outlines the method for the production of heterologous proteinaceous substances in protonema tissue in accordance with claims 1-6, 17 and 19-21 of the present invention, as described on page 8, line 1, to page 18, line 35, using known and readily available materials. The crux of the present invention is obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The method in accordance with these directions may require some experimentation in order to optimize results using protonema from species other than *Physcomitrella patens*, *Ceratodon purpureus* and *Marchantia polymorpha*; however, Applicants assert that while some experimentation may be necessary, it is no more than is commonly encountered in the art. Applicants' assertion is supported by the experimental evidence submitted in the Gorr Declaration, which shows successful transformation of *Funaria hygrometrica* was achieved using the protocol described on page 14, line 1, to page 16, line 7,

and on page 16, lines 28-35, of U.S. Patent Application No. 10/089,450, and that such successful transformation required no more than routine experimentation as is commonly encountered in the art (Reski Declaration, ¶¶ 36-38; Gorr Declaration, ¶¶ 15, 16 and 21-23).

As is clearly established by the scientific references of record and by the testimony and experimental evidence provided by the Gorr Declaration and the Reski Declaration, a person of ordinary skill in the art would know how to transform *Physcomitrella patens*, *Ceratodon purpureus*, *Funaria hygrometrica* and *Marchantia polymorpha* protoplasts, in view of Applicants' disclosure and the state of the art, without undue experimentation. However, transformation of plant protoplasts is not the crux of the invention. Be that as it may, transformation of other plant protoplasts of species that produce protonema tissue is a matter of routine experimentation in the art in view of the biological predictability of protonema forming plant tissues in general.

Thus, as Dr. Reski has concluded, the seventh Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

7. The Claims Are Not Overbroad

Seventh, the breadth of claim 1 includes the method of production of heterologous proteinaceous substances in protonema tissue. The term "protonema" has a specific meaning in the art and limits the scope of the present invention to tissues that are "the primary usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts." Furthermore, the claimed method includes two steps: (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The breadth of claim 1 includes the species embodiment disclosed on page 11, line 29, to page 27, line 26, and is limited to transformed bryophyte strains. The breadth of claim 1 is not overly broad, as Dr. Reski has testified (Reski Declaration, ¶ 39).

Thus, as Dr. Reski has concluded, the eighth Wands factor weighs in favor of enablement (Reski Declaration, ¶49).

Summary of the Wands Factors Applied to the Protonema Tissue Embodiment

Application of the facts to the *Wands* factors demonstrates that, as a matter of law, Applicants' application as originally filed would teach a person of ordinary skill in the art how to make and use the claimed invention without undue experimentation. Specifically, Applicants' application provides (i) considerable direction and guidance on how to practice the invention, and the methods and materials needed to practice the invention are well known, (ii) at least one very detailed working example of protonema tissue producing heterologous proteinaceous substances, (iii) that the nature of the invention pertains to simple, predictable plant organisms, (iv) that the state of the are is mature, and (v) that there is an extremely high level of skill in the art. While the degree of predictability in practicing the invention is not 100% (i.e., because not every possible protonema forming species has been studied in the prior art), multiple species of mosses and liverworts have been transformed in the prior art and by the disclosure of the present invention. Furthermore, the experimental evidence of the Gorr Declaration shows how easy it is to transform additional bryophyte species besides *Physcomitrella patens*, using no more than routine experimentation, thereby demonstrating (vi) a high degree of predictability in the art. With respect to the transformation of bryophytes, the predictability is reasonably more predictable than other biotechnological arts that have been deemed enabled under similar circumstances. See In re Wands, 8 U.S.P.Q.2d at 1406. (vii) The quantity of experimentation necessary to practice the invention, although not zero, is not undue. Lastly, (viii) the breadth of the independent claims 1, 17 and 19 of the present invention covers, in scope, the genus (in the patent law sense) of protonema tissue taught in the specification. The breadth of claim 1 is not overbroad.

In short, all of the Wands factors favor enablement.

E. Applicants' Rebuttal of the Examiner's Interpretation of the Wands Factors

Analyzing the same Wands factors as Applicants, the Examiner arrived at an opposite conclusion (March 14th Office Action, at 2, line 9, to at 8, line 26). The Examiner's analysis of the Wands factors is flawed, however, because the Examiner has considered each factor singly and deemed each factor alone insufficient to establish enablement when a proper enablement analysis requires consideration of all of the Wands factors taken together as a whole. In re

Wands, 8 U.S.P.Q.2d at 1404. The Examiner's treatment of the Wands factors as a series of single, simple, unrelated factual determinations is incorrect as a matter of law. Id.

The Examiner's analysis is additionally flawed because the Examiner has misconstrued, and mischaracterized, the claims and the following Wands factors. First, the Examiner insists, as discussed above, that the step of "transformation of mosses and liverworts [is] within the full scope of the claims" (Office Action, dated March 14, 2006, at 4, lines 1-3). The step of "transformation" is not recited anywhere in the claims. The Examiner is plainly reading limitations from the specification into the claims, which is not permitted. Texas Digital Systems Inc. v. Telegenix Inc., 64 U.S.P.Q.2d at 1819.

Second, the Examiner contends that "transformation of mosses and liverworts is not taught within the full scope of the claims," and asserts that the specification fails to teach how to transform *C. purpureus* or *M. polymorpha* (Office Action, dated March 14, 2006, at 5, lines 15-18). The Examiner goes on to assert that "'the specification, not the knowledge of one skilled in the art' must supply the enabling aspects of the invention," citing Genentech Inc. v. Novo Nordisk A/S, 42 U.S.P.Q.2d 1001, 1005 (Fed. Cir. 1997). This quotation is incorrect and incomplete. The quotation actually reads "the specification, not the knowledge of one skilled in the art, must supply the *novel* aspects of the invention in order to constitute enablement." Id. (emphasis added).

The invention in Genentech pertained to the application of cleavable fusion expression to make hGH; however, the applicants did not disclose any starting materials or any reaction conditions under which cleavable fusion expression would work. Genentech, 42 U.S.P.Q.2d at 1004. In other words, the applicants in Genentech provided no working example. This is much different from the present case where, as conceded by the Examiner, there is a working example of transformation of a bryophyte species (Office Action, dated March 14, 2006, at 3, lines 4-6). Thus, starting material and reaction conditions are explicitly described in the specification of the above-captioned application (See Specification, page 11, line 30, to page 18, line 35). Other starting material mentioned in Applicants' specification include mosses and liverworts of the genus *Physcomitrella, Funaria, Sphagnum, Ceratodon, Marchantia* and *Sphaerocarpos* (See Specification, page 7, lines 1-6).

The Examiner also ignores that the Court in Genentech acknowledges the well-established rule that "a specification need not disclose what is well known in the art." Genentech, 42 U.S.P.Q.2d at 1005. In fact, what the Genentech Court said with respect to the Examiner's proposition is

> omission of minor details does not cause a specification to fail to meet the enablement requirement. However, when there is no disclosure of any specific starting material or of any of the conditions under which a process can be carried out, undue experimentation is required...It is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement.

Id.

In this case, methods of transformation of *Physcomitrella, Ceratodon* and *Marchantia* are well known in the art as shown by the Reutter article; M. Zeidler et al., *Transgene expression in the moss Ceratodon purpureus.* 154 J. Plant Physiol. 641, 641-650 (1999) (hereinafter, the "Zeidler article," of record); the Nasu article; and as conceded by the Examiner. Thus, the omission of such minor details from Applicants' specification does not create a tenable issue regarding lack of enablement, as the Examiner contends.

Because Applicants' specification provides adequate direction and guidance as emphasized by Dr. Reski's testimony (Reski Declaration, ¶¶22-25), and a working example with starting materials and reaction conditions described (Reski Declaration, ¶ 26) as conceded by the Examiner (Office Action, dated March 14, 2006, at 3, lines 4-6), the rule acknowledged by the Genentech Court applies wherein the omission of minor details well known in the art does not cause a specification to fail to meet the enablement requirement. The proposition asserted by the Examiner is untenable because it relates to a quote taken out of context from the Genentech decision and simply does not apply to the facts of this case.

Third, the Examiner has failed to acknowledge that expert testimony, such as in the present case, may be used to establish enablement. H.H. Robertson Co. v. United Steel Deck, Inc., 2 U.S.P.Q.2d 1926, 1928 Fed. Cir. 1987), *overruled on other grounds by* Markman v. Westview Instruments, Inc., 34 U.S.P.Q.2d 1321 (Fed. Cir. 1995). In this case, Dr. Reski, a world expert in the art, has provided testimony in support of enablement of the presently claimed invention (Reski Declaration, ¶¶ 21-40 and 49).

Fourth, the Examiner contends that Dr. Gorr is a person of extraordinary skill in the art (Office Action, dated March 14, 2006, at 6, lines 21-22, and at 7, lines 17-19). Whether or not Dr. Gorr is a person of extraordinary skill is irrelevant, since he is an expert and is competent providing his expert opinion as to what can be done by one of ordinary skill in the art. The same is true of Dr. Reski. The Examiner has not provided any evidence as to the skill in the art to overcome the evidence of record. The Examiner's bare and unsupported conclusions are not supported by any evidence.

Fifth, the Examiner rejects the experimental evidence provided by the Gorr Declaration, demonstrating the ease with which other bryophyte species such as *Funaria* may be transformed, on the grounds that such experimental evidence is not taught in the specification, does not represent the full scope of the claims, and because Dr. Gorr is a person of extraordinary skill in the art (Office Action, dated March 14, 2006, at 6, lines 10-22). The Examiner's contention ignores the rule that permits experimental testing by an expert to demonstrate enablement by showing that the invention claimed may be practiced without undue experimentation. Bruning v. Hirose, 48 U.S.P.Q.2d 1934, 1939 (Fed. Cir. 1998). As stated above, whether Dr. Gorr is of extraordinary skill in the art is irrelevant, he is competent to give his opinion on what one of ordinary skill in the art is capable, and his evidence is uncontroverted in the present case.

In this case, Dr. Gorr has demonstrated that the protocols described in Applicants' specification may be successfully applied to other bryophyte species besides *Physcomitrella*, such as *Funaria* and *Marchantia*, without undue experimentation (Gorr Declaration, ¶¶ 14-23). Applicants have provided direct evidence of applicability of the present invention to multiple bryophyte species without undue experimentation. Dr. Gorr's evidence supports the proposition that the presently claimed invention is broadly applicable to mosses and liverworts without undue experimentation. Dr. Gorr's evidence also supports the proposition that the present invention utilizes plant material that is predictable in its biological transformation characteristics. These reasonable inferences all support the conclusion that Applicants' claimed invention is adequately enabled by the originally filed disclosure.

For all of the above reasons, Applicants have demonstrated that the invention of claims 1-6, 17 and 19-21 is adequately enabled by Applicants' disclosure as originally filed.

II. THE REJECTION UNDER 35 U.S.C. § 103(a) IS UNTENABLE

A prima facie case of obviousness requires a showing that the scope and content of the prior art teaches each and every element of the claimed invention, and that the prior art provides some teaching, suggestion, or motivation to combine the references to produce the claimed invention. In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992); In re Vaeck, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). In the present case, the Examiner has failed to establish a prima facie case of obviousness because the references do not teach, or suggest, all of the claimed elements and the references fail to provide a teaching, suggestion, or motivation justifying the asserted combination, particularly in view of the present amendments to the claims.

The Examiner states at least a half-dozen times that "one cannot show nonobviousness by attacking references individually," citing *In re Keller*. Applicants are in fact saying that certain claim elements are not present in the references. If the combination of references applied by the Examiner does not have each and every element of the claims, a *prima facie* showing of obviousness is not shown. In re Oetiker, 24 U.S.P.Q.2d 1443. Notably, none of the cited references teach, or even suggest, obtaining secreted heterologous proteinaceous substances from intact protonema tissue, which is made up of cells having cell walls.

A. The Prior Art Does Not Teach the Elements of the Claims

The Houba-Hérin Article Does Not Teach Any Element of the Claims Despite the Examiner's Reliance Thereon

The Houba-Hérin article is directed merely at confirming enzyme activity following short-term, transient expression in protoplasts lacking cell walls. It does not relate to protonema tissue as recited by the claims. Furthermore, the authors themselves admit they do not know whether the protein of interest was liberated by cell lysis or was secreted. As discussed in more detail below, the article is quite inappropriate for use in a rejection under § 103. The Examiner cannot rely on the reference to show a fact, such as protein secretion, when the authors themselves admit it is not established.

The Houba-Hérin Article Relates Only to Protoplasts, Which Lack Cell Walls

The Houba-Hérin article teaches transforming moss protoplasts, in particular *Physcomitrella patens* protoplasts, to analyze cytokinin oxidase (CKO) or β-glucuronidase (GUS) activity (Houba-Hérin article, See Abstract, and page 619, col. 2, lines 21-48, and page 624, col. 2, lines 35-53). Protoplasts are single cells lacking cell walls, unlike the protonema tissue recited by the claims, which is inherently multicellular and with the cells having cell walls.

Transiently transformation of protoplasts as taught by the Houba-Hérin article (which doesn't teach transformed protonema tissue) and keeping protoplasts alive for a short period of time (44 hours) would neither result in cell division requiring cell walls nor protonema tissue, as recognized by anyone skilled in the art. Reski Decl., ¶ 25. (See also Webster's new collegiate dictionary 927 (1977), "**protoplast**...**2 a :** the nucleus, cytoplasm, and plasma membrane of a cell constituting a living unit distinct from inert walls and inclusions"). On the other hand, the present invention obtains heterologous proteinaceous substances secreted from "protonema tissue" as recited in claims 1, 17 and 19, which one skilled in the art would recognize are differentiated plant tissues (See Webster's new collegiate dictionary 927 (1977) "**protonema**...**:** the primary...thalloid stage of the gametophyte in mosses"). A person of ordinary skill in the art would know that such protonema tissue is made up of cells having cell walls.

While the present inventors may make use of plant protoplasts when transforming cells, the present invention uses transformed protonema tissue for producing the heterologous proteinaceous substances comprising transit peptide, and it is the heterologous proteinaceous substances produced by the protonema tissue that are obtained without disrupting producing protonema tissue or cells. Thus, while plant protoplasts may be used in preparing transformed protonema tissue for practicing the presently claimed invention, it is protonema tissue that is used in accordance with the present invention for producing the heterologous proteinaceous substances. Thus, plant protoplasts are not an element of the present invention as claimed.

While the present invention includes the step of culturing transformed protonema tissue, and the present invention uses the transformed protonema tissue for producing the heterologous proteinaceous substances, the steps of transforming protoplasts and growing them into transformed protonema tissue are not elements of the claims. Thus, while plant protoplasts may be used in preparing transformed protonema tissue for practicing the presently claimed

invention, it is culturing transformed protonema tissue that is presently claimed as a step in Applicants' "method for the production of heterologous proteinaceous substances in plant material". The process of transforming plant protoplasts and of using transformed plant protoplasts to generate transformed protonema tissue is not an element of the present invention as claimed.

The Examiner baldly refers to "Applicant's error in interpretation of what Houba-Hérin et al. mean when discussing the cell wall" (Office Action dated March 14, 2006, pages 11 and 13 referring to page 10) without citing any support for the supposed error. On referenced page 10 the Examiner discusses glycosylation status of the protein, which is irrelevant to the claims.[1] Moreover, the Houba-Hérin authors admit that relationship between glycosylation and functionality and/or localization "requires further investigation." Page 621, second column, last paragraph. Applicants have made no error – the Houba-Hérin article simply does not disclose expression in cell-wall containing material, as well discussed above. The contents of paragraph 24 of the Gorr declaration are accurate. The Examiner provides no evidence or facts to the contrary, and Applicants vehemently dispute the Examiner's assertion that the Gorr declaration contains any misrepresentation.

The Examiner also misrepresents the facts by stating "Houba-Hérin et al. suggest producing the enzyme in a moss system in order to get proper processing of the enzyme; this would require secretion (pg 621, right column, paragraph 2)." The reference contains no allegation that proper processing would require secretion. Altough it is correct that a protein needs to be introduced into the "secretory pathway" in order to be glycosylated, this does not mean that the glycosylated protein will be secreted into the outer space, i.e., into the culture medium. The Examiner cannot cite any requirement for secretion in order to obtain proper processing of the enzyme, and this is not at all mentioned in the reference. Why would a cell go through the trouble of properly producing an enzyme in order to secrete it? Proper processing simply does not require secretion.

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[1] The Houba-Hérin article discusses (at the top of p. 621, second column) why they selected the moss system. There had been previous reports of CKO's with sizes of 70-78 kD and 68 kd respectively. This was however not in agreement with the result of the native enzyme with gel filtration and the use of FPLC columns (44 kDa) (see p. 620). This was suspected to be a glycosylation artifact. Houba-Hérin tried expression of recombinant CKO in *E. coli* without success. For that reason they decided to analyze the functionality of the enzyme in that a plant system that would most likely process the enzyme.

In summary, the Houba-Hérin article does not disclose or suggest the either of the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells.

The Houba-Hérin Article Does Not Teach Secreted Proteins

The Houba-Hérin article fails to teach obtaining secreted proteins, as required by the present claims. The authors themselves admit they **do not know** whether the protein of interest was liberated by cell lysis or was secreted. (Houba-Hérin article, at 621, col. 2, lines 10-12).

Applicants file herewith the declaration of Klaus von Schwartzenberg, a scientist who worked in the same facility as the cited reference and who is an expert in the field. As explained in the declaration of Dr. Schwartzenberg, ¶¶ 4-5, the methods Houba-Hérin article could result in lysis of the protoplasts during centrifugation, so that intracellular material would be found in the supernatant. Moreover, the unavoidable, if perhaps low-level, cell lysis during culture would release some amount of the protein into the medium. The Houba-Hérin article simply does not teach obtaining secreted proteins.

Also, according to Dr. Reski's testimony (Reski Declaration, ¶ 44), the Houba-Hérin article employs a method for detecting enzyme (i.e., cytokinin oxidase (CKO)) that is both indirect, because it pertains to detecting levels of CKO enzyme activity rather than the enzyme itself, and that is directed to detecting trace levels of enzyme as evident from the photolabeling of cytokinin oxidase (CKO) with [^{3}H]-azidoCPPU (See Houba-Hérin article, at 616, first col., line 26, to second col., line 2). In Dr. Reski's opinion, a person of ordinary skill in the art employs such a photolabeling detection method in place of conventional methods of direct measurement, such as the ELISA methods described on page 20, lines 21-27, and page 27, lines 11-26, of the above-captioned application and the ELISA method employed by Dr. Gorr, when the amount of detectable protein is expected to be in trace amounts, if any is produced at all (Reski Declaration, ¶ 44). As Dr. Reski has testified, "[t]he Houba-Hérin article also does not teach the application of a transit peptide, such as is included in the present claims. (Reski Declaration, ¶ 44, emphasis added) (See also the Declaration under 37 C.F.R. § 1.132 by Dr.

Klaus von Schwartzenberg, filed herewith, and hereinafter referred to as the "Schwartzenberg Declaration," ¶¶ 5-7). As taught by Armin Baur et al., *A fast and flexible PEG-mediated transient expression system in plants for high level expression of secreted recombinant proteins*, 119 J. Biotechnology 332, 337 (2005) (of record), a target protein that is not associated with a signal peptide is not likely to be secreted in significant amounts from moss protoplasts. According to Dr. Reski, this fact would be even more true of moss protonema, which have a cell wall acting as a barrier further limiting secretion (Reski Declaration, ¶ 44). In view of these facts, it is Dr. Reski's opinion that a person of ordinary skill in the art would have no motivation to apply the teachings of the Houba-Hérin article regarding the formation of transiently transformed moss protoplasts for any useful or commercial purpose because the yields of the CKO enzyme are, at most, trace amounts (Reski Declaration, ¶ 44).

Thus, the Houba-Hérin article teaches neither protonema tissue, nor producing heterologous proteinaceous substances from a construct with a signal peptide operably linked to a protein, nor secreted proteins, each as required by the present claims.

The Reutter Article

The Reutter article teaches the transformation of moss protoplasts, in particular *Physcomitrella patens* protoplasts, using PEG-mediated direct DNA transfer with a 11.5 kb plasmid carrying the E. *coli gus*-gene under the control of the cauliflower mosaic virus (CaMV) 35S-promotoer as well as the *npt*II-gene under control of the *Agrobacterium tumefaciens* nopalin synthase promotor (Reutter article, page 143, line 4, to page 144, line 5). The Reutter article also discloses culturing protonema to form protonema balls using high speed stirring (Reutter article, at 145, lines 1-5). However, the Reutter article is completely silent with respect to obtaining the β-glucuronidase produced by the moss protonema balls without disrupting producing tissues or cells as recited in claims 1, 17 and 19. It can be inferred from the teachings of the article that any detected material was from lysed or disrupted cells. The purpose of Reutter's study was not to obtain heterologous proteinaceous substances from culture media and there is no indication in their study that this was achieved or even possible.

The Reutter article explicitly used an assay taught by Jefferson to quantify intracellular β-glucuronidase activity of the transformed moss plants, which necessarily involved the lysis of the transformed cells (See Reutter article, at 143, lines 23-32; and Richard A. Jefferson, *Assaying chimeric genes in plants: the GUS gene fusion system*, 5 Plant Molecular Biology Reporter 387, 392 (1987) (of record). Thus, there is no teaching in Reutter that isolation of heterologous proteinaceous substances from medium was possible, and the assay they used specifically indicates that they did not believe it was possible.

In addition, Dr. Reski testifies that, as co-author of the Reutter article, he knows the stably transformed *Physcomitrella* protoplasts of the Reutter article expressed a heterologous protein that was localized in the cells (Reski Declaration, ¶ 46). Dr. Reski testified, as co-author of the Reutter article, that no secretion of the heterologous protein into the medium was observed from stably transformed protonema because for secretion of the heterologous protein to occur there has to be at least a signal peptide (i.e., a transit peptide) included with the heterologous protein, and the method taught by the Reutter article did not employ a signal peptide (Reski Declaration, ¶ 44). Consequently, the Reutter article neither teaches, nor suggests, there would be secretion of heterologous protein through the cell wall of protonema cells.

It is thus evident that the Reutter article does not disclose or suggest either of the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells.

For all of these reasons, Reutter cannot support any rejection of the claims under § 103.

The Nasu Article

The Nasu article pertains to the transformation of *Marchantia polymorpha* that is a haploid liverwort with very small genome DNA (See Abstract). In particular, the Nasu article teaches transforming single *Marchantia* cells with a binary vector plasmid pB1121 and a plasmid pRiA4b so as to inactivate the GUS gene (Nasu article, at 520, first column, lines 1-11). Thus, the Nasu article pertains to cultures of single cells, and not to intact plant tissue. The transformed cells were subsequently fixed in formaldehyde, which a person of ordinary skill in

the art would realize kills the cells, and then the dead cells were stained for GUS activity (Nasu article, at 520, first column, lines 35-47, and see Figure 4).

The above analysis of the Nasu article is supported by the testimony of Dr. Gorr, who states that the Nasu article does not teach that heterologous protein produced by transformed *Marchantia* cells would be secreted through the cell walls of mature protonema cells (See Gorr Declaration, ¶ 28). Dr. Reski also testified that the Nasu article does not teach, or suggest, the application of a signal peptide, and therefore, also neither teaches, nor suggests, there would be secretion of heterologous protein through the cell wall of protonema cells (Reski Declaration, ¶ 46). Thus, the Nasu article plainly does not teach or suggest either of the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells.

For all of these reasons, Nasu cannot support any rejection of the claims under § 103.

The Raskin Patent

The Raskin Patent teaches "methods for recovering polypeptides from higher plants and portions thereof," which involves using plants to produce and exude heterologous polypeptides (See Abstract). The polypeptides recovered by the Raskin method are obtained by collecting plant exudates, which is fluid such as guttation fluid that "oozes out" of certain structures of higher order plants such as roots and leaf hydathodes (col. 4, lines 12-15 and lines 57-63). However, rhizosecretion and guttation require specialized plant structures that are lacking in moss and liverwort plant protonema (See Reski Declaration, ¶ 47). Protonema have no roots (See, e.g., Physcomitrella reference of record) and instead are photoautotropic tissue (Reski Declaration, ¶ 47). Without roots, protonema are not capable of rhizosecretion. Protonema also do not have hydathodes, which are a particular variant of stomata (leaf opening). When exposed to copious watering and high air humidity, higher order plants that have roots will exude water through special leaf tip cells (i.e., hydathodes in the leaves) forming drops of water in the process known as guttation (See en.wikipedia.org/wiki/Guttation, last downloaded September 9, 2006, one page, attached herewith). Because root pressure provides the impetus for guttation fluid

flow through the vessel system of the plant and because rhizosecretion requires a root structure, it is Dr. Reski's expert opinion that a person of ordinary skill in the art would have no motivation to apply the teachings of the Raskin Patent, which pertains to higher order plants having vessels and roots, to plants such as mosses and liverworts, which neither have vessels nor roots (Reski Declaration, ¶ 47).

Contrary to the Examiner's assertion (Office Action of March 14, 2006, page 14, lines 15-19), Raskin does **not** generally teach isolation of heterologous proteins secreted from plants. Raskin detected protein in samples from root **exudates** and root **intercellular fluid**. Col. 13, lines 1-49. Because protonema lack roots, and because the claims are directed at secreted material, the teaches of the reference are not applicable to the present invention. Highly differentiated plant roots are not comparable to the protonema of the claims.

For all of these reasons, Raskin cannot support any rejection of the claims under § 103.

The Stuart Patent

The patent to W. Dorsey Stuart (U.S. Patent No. 5,776,730) (hereinafter "Stuart"), although not cited in a rejection, is inappropriate art for several reasons.

First, it relates to the fungus *Neurospora* and cannot be compared to the plant genus protonema tissue of the present invention. Fungi are classified in a completely different kingdom of organisms from the plants of the present invention, and moreover are heterotrophic requiring utterly different culture conditions. These massive differences from the subject matter of the claims indicate no motivation to combine with any teachings regarding the claimed protonema.

Second, the reference describes refers to "host cells" and not tissue, as claimed in the present invention. At no time does the Stuart patent mention "tissue." One of skill in the art would expect recovery of secreted proteins from intact tissue to be significantly more difficult than from individual cells.

Moreover, fungi normally produce high quantities of extracellular proteases. This is a great disadvantage if one wishes to produce heterologous, recombinant proteins, because the proteins would be degraded.

Even if Stuart taught obtaining protein from individual funal cells with cell walls (not photoautotrophic tissue as claimed here), the reference is not enabling for what the Examiner asserts it teaches. The Examiner asserts that producing secreted proteins in cell-wall containing organisms is well-known in the art (Office Action dated March 14, 2006, page 12, lines 3-4), but this is false. Stuart provides only a single fungus of *Neurospora* as an example, which by any standard does not enable practicing the teaching in all tissues with cell walls, including plants. The reference is even less enabling under the extraordinarily stringent standard the Examiner has applied to Applicants. A patent or printed publication does not disclose an invention for prior art purposes if the disclosure is not enabling. In re Donohue, 766 F.2d 531, 533 (Fed. Cir. 1995). Even if Stuart were enabled with regard to secretion of proteins from the fungus *Neurospora*, the reference is absolutely not enabled with regard to the same for cell-wall-containing cells in general, much less cell-wall-containing plant tissues.

For all of these reasons, Stuart cannot support any rejection of the claims under § 103.

The Radin Patent

The patent to Radin et al. (U.S. Patent No. 5,929,304) (hereinafter "Radin"), although not cited in a rejection, is also inappropriate, because it teaches disrupting plant material and never secretion from intact tissue as presently claimed.

This reference relates to the production of lysosomal enzymes in plant expression systems, and is directed at solving the problem of pathway engineering, namely expression in plants, which have vacuoles, as opposed to mammalian cells, having lysosomes. Radin, col. 6, line 51 to col. 7, line 8. With respect to applicable plant systems, Radin refers to all of the botanical world (column 16, lines 12-22), without mentioning moss. Only tobacco is exemplified. Claims were issued without limitation to any particular plant system.

The reference then talks about transformed plants, plant cells and plant tissues (column 7, lines 12-13), out of which the desired enzymes can be isolated (i.e., they are not excreted) (column 7, lines 22-24). The invention of the reference is exemplified by transgenic tobacco plants with three particular lysosomal enzymes (column 7, lines 24-33). Several experiments and parts of the description relate to experiments with extracts that are obtained by destruction of the plant tissue. The promoter used is the so-called MeGA promoter, which stands for

mechanical gene activation (MGA) (column 8, lines 34-35). The meaning of this is described at column 9, line 6: "hrs. post induction (i.e., wounding of tissue or MGA)." *See also* column 10, lines 7-11 and figs 14 A and B with regard to induction of IDUA transgene in tobacco leaf tissues wherein leaf tissue from transgenic plant IDUA-9 was induced by excision into 1.5 mm strips and incubated at room temperature on moist paper towels in sealed plastic bag. This form of induction destroys the substance producing tissue. Naturally, sequestered substances can, under these conditions (destroyed tissue, sealed culture and very high humidity, allowing guttation) be released and/or leach into the incubation buffer. Various cases of activity in the incubation buffer were found in which cut of leaves (cut into strips) were induced and incubated (in incubation buffer in a closed system with high humidity). At best, Radin's alleged secretion is no more than the guttation of Raskin discussed above, but it also entails substantial cellular lysis from destroyed tissue. At no time does Radin disclose or enable secretion from intact tissue as presently claimed.

Although a series of possible induction methods are referred to, the only thing actually disclosed is wounding or other mechanical gene activation (MGA) column 18, lines 2-10. Radin admits that when "the lysosomal enzyme is targeted for localization within the plant cell, the plant cell wall must be penetrated to extract the protein. Accordingly **the plant tissue can be ground to a fine powder**, e.g., for example by using a tissue grinder and dry ice, or homogenized with a ground glass tissue homogenizer." Col. 18, lines 16-20. Thus much of the reference relates to results obtained with cell extracts. The discovery that proteins are discovered in the incubation buffer results from the usage of induction of the MeGA promoter by the use of slicing. In example 6.2.4., leaf slices of plants are "**harvested (and thereby wounded)** in order to induce transgenic expression. " Column 24, lines 39-44. Later, an extract is studied. Examples 6.2.5. to 6.4 relate only to extracts of previously wounded leaf pieces. The proof of the IDUA protein is described in 7.4.1. In the first part, it relates to extracts of uninduced or induced plant tissue (column 30, lines 11-41). In order to achieve expression, leaves are harvested, induced by mechanical wounding, and incubated at RT and high humidity (i.e., the wounded leaves are wrapped in moist filter paper in sealed bags or layered in a container with buffer gently swirled over the tissue). Column 30, lines 43-48. In addition from the proof obtained from extracts, later experiments show that IDUA came out during the incubation of the

(wounded) leaves and could be detected on the filter paper. This is called "leaching out" and has nothing to do with the secretion of an undamaged autonomic tissue in the context of a continuous process for the production of heterologous proteins!

Other possibilities of induction without wounding or cutting up the tissues are not disclosed. Signal peptides of the sort described in the present invention are mentioned as an optional alternative, column 14, lines 31 to column 15, line 9, where Radin proposes transport of protein to the plant vacuole, and a construct "engineered to target the lysosomal enzyme for secretion." Alternatively such peptides are mentioned because their proteins are retained in the ER. There is no concrete disclosure of any actual secretion, which is not surprising since the plants are induced by wounding, or cutting in strips. To assert that Radin teaches secretion from intact plant tissues is mere wishful handwaving.

Throughout Radin, protein production and subsequent recovery occurs following physical wounding (the mechanical gene activation), and tissue is "**ground to a fine powder**," (col. 18, lines 9-18). Another example uses harvested leaves that leach out material through wounds. Col. 30, lines 42 - 54. This is a far cry from the present invention, with claims requiring obtaining protein "without disrupting producing tissues or cells". Radin never teaches, or even suggests, obtaining secreted heterologous proteinaceous substances from intact tissue, as presently claimed.

The leaching-out from harvested plant parts (leaves) into buffer does nothing to reduce the nonobviousness the present invention. The leaves of Radin are destroyed in order to produce protein. **The present invention claims recovery of secreted material "without disrupting producing tissues or cells." Radin fails to teach secretion from plant material that has not been wounded.**

Moreover, by the Examiner's own standard, the reference is not enabled for plants other than tobacco, nor for actual secretion. Radin only provides tobacco as an example and does not enable producing proteins in cell-wall containing organisms in general. Although a series of possible induction methods are referred to, the only thing actually disclosed is wounding or other mechanical gene activation (MGA) column 18, lines 2-10. The Examiner asserts that secreted proteins in cell-wall containing organisms is well-known in the art (Office Action dated March 14, 2006, page 12, lines 3-4), but this is false. A patent or printed publication does not disclose

an invention for prior art purposes if the disclosure is not enabling. In re Donohue, 766 F.2d 531, 533 (Fed. Cir. 1995). Radin certainly is not enabled for secretion from intact tissues having cell walls.

For all of these reasons, Radin cannot support any rejection of the claims under § 103.

B. Summary of the Prior Art

In summary, the Houba-Hérin article teaches transiently transforming and maintaining *Physcomitrella patens* protoplasts for a short period of time in order to analyze cytokinin oxidase (CKO) or β-glucuronidase (GUS) activity, but it does not teach (i) culturing transformed protonema tissue, transformed with a construct encoding a signal peptide operably linked to a protein, and (ii) it does not teach obtaining secreted heterologous proteinaceous substances from protonema tissue without disrupting producing tissues or cells.

The Reutter article teaches the stable transformation of *Physcomitrella patens* protoplasts using the PEG-mediated direct DNA transfer in order to intracellularly accumulate β-glucuronidase. While the Reutter article teaches culturing moss protonema and protonema balls, the article is completely silent with respect to obtaining the β-glucuronidase produced by the protonema without disrupting producing tissues or cells. And in fact, the only assay used for the testing for β-glucuronidase was one that required the lysing of cells (i.e., the complete disruption of the cells), which indicates that the authors did not believe that isolation of the β-glucuronidase from the culture medium was possible. The Reutter article also does not teach, or suggest, producing heterologous proteinaceous substances and transit peptide, and especially does not teach or suggest obtaining secreted heterologous proteinaceous substances from protonema tissue without disrupting producing tissues or cells.

The Nasu article teaches transforming *Marchantia* cells and analyzing recombinant intracellular GUS activity by fixing plant material in formaldehyde and staining for GUS activity. The Nasu article does not teach production of extracellular heterologous proteinaceous substances, it does not teach production of transit peptide, and it does not teach obtaining secreted heterologous proteinaceous substances without disrupting producing tissues or cells.

The Raskin Patent pertains to using plants or intact portions thereof, which includes a root structure, so that heterologous polypeptides can be exuded by root structures or hydathodes

of higher plants, and then collected from the exudates. However, the method taught by the Raskin Patent is relevant to only higher order plants having vessels and the necessary root structure required for rhizosecretion and/or guttation. A person of ordinary skill in the art would have no expectation that the Raskin method could be applied to protonema, which have no vessels and no root structure.

Although Stuart and Radin are not cited in a rejection, they do nothing to reduce the nonobviousness of the present invention. The Examiner has cited the references for the proposition that secreted proteins in cell-wall containing organisms is well-known in the art, but for all of the reasons discussed above, this is simply not true.

Plainly, none of these prior art references reasonably teach, or even suggest, either of the steps, in accordance with claims 1, 17 and 19 of the present invention, of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The scope and content of the references is, therefore, insufficient to establish a prima facie case of obviousness.

To be absolutely clear regarding this point, all combinations of the Houba-Hérin article, the Reutter article, the Raskin Patent, and the Nasu article would still fail to teach, or suggest, the steps recited in each of the independent claims. For the above reason alone, the Examiner's § 103 rejection is untenable and must be withdrawn.

C. Lack of Proper Motivation to Combine or Reasonable Expectation of Success

A proper rejection under Section 103 further requires showing (1) that the prior art would have suggested to a person of ordinary skill in the art that they should make the claimed device or carry out the claimed process, (2) that the prior art would have revealed to a person of ordinary skill in the art that in so making or doing, there would have been a reasonable expectation of success, and (3) both the suggestion and the reasonable expectation of success must be found in the prior art and not in the applicants' disclosure. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). In the present case, the Examiner has not shown that the references of record provide a suggestion to combine the references, or that combination of the references would lead to a reasonable expectation of success.

Applicants point out that a person skilled in the art could not have predicted that using protonema tissue would be a good approach for easily obtaining heterologous proteinaceous substances from the medium for the reason that the producing protonema tissue comprises cells having cell walls (Reski Declaration, ¶¶ 45-46; Gorr Declaration, ¶ 27). Typically, proteinaceous substances made by fully differentiated plants are trapped in a space located between the plasma membrane and the cell wall. Consequently, conventional retrieval of such substances from mature plants requires disrupting their cell wall, e.g. by mechanical or chemical means. However, in accordance with the invention as recited in claims 1, 17 and 19 and as supported by the evidence of record, the "protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein" enables "obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells." For this reason, one of ordinary skill in the art would have a poor expectation of success to try and obtain protein secretion from tissue having cell walls, and thus no motivation to combine elements of the prior art.

As discussed above, the Houba-Hérin article teaches a method of transiently transforming and maintaining moss protoplasts for a short period of time, and does not teach, or suggest, a method for the expression and secretion of recombinant proteins in liquid culture medium using protonema tissue. More particularly, it fails to suggest any means of obtaining secreted proteins from tissue made up of cells having cell walls. As explained by the testimony of Dr. Schwartzenberg, an expert in the field of plant cell cultivation/development research and transformation, the Houba-Hérin article is limited to demonstrating the transient expression of CKO enzyme in moss protoplasts in order to demonstrate the functionality of the recombinant enzyme (Schwartzenberg Declaration, ¶¶ 5 and 7). As also established by Dr. Schwartzenberg's testimony (Schwartzenberg Declaration, ¶¶ 4-7), a person of ordinary skill in the art would realize that the Houba-Hérin article does not teach, or suggest, a method for the expression and secretion of recombinant proteins in liquid culture medium because the article is silent on the subject of a signal or leader peptide such as is necessary for protein secretion. Dr. Schwartzenberg's considered opinion is that "it would be entirely unsupported speculation to say that the CKO activity definitely represented extracellular protein and not protein released from cells." (Schwartzenberg Declaration, ¶ 5).

The Reutter article teaches a method of transforming moss protoplasts that are regenerated to the protonema stage in order to continuously produce an intracellular heterologous protein. The Houba-Hérin and Reutter references do not teach how such different protocols could be combined, and the references provide no motivation for the combination as supported by the testimony of Dr. Gorr (See Gorr Declaration, ¶¶ 26 and 29). Furthermore, the references do not provide one of ordinary skill in the art with a reasonable expectation of success even if the combination was made because neither reference teaches the production of a "transit peptide" in accordance with the present invention.

Even assuming, *arguendo*, that the proposed combination of the teachings of the Houba-Hérin article and the Reutter article could be made (which Applicants assert is an erroneous assumption), it is a fact that this combination would clearly fail to teach, or suggest, a reasonable expectation that the heterologous protein could be obtained without disrupting producing tissues or cells because neither reference teaches a transit peptide, and protonema have cell walls that serve as a barrier to secretion (Reski Declaration, ¶¶ 45-47; Schwartzenberg Declaration, ¶¶ 5-7)[2]. The teachings of the Nasu article would fail to make up any of these deficiencies. The Raskin Patent cannot be combined with the teachings of any of the Houba-Hérin article, the Reutter article, and the Nasu article because the Raskin Patent pertains to plants having roots and vessels, as are needed for rhizosecretion and guttation, whereas the three articles pertain to bryophytes, which have no vessels and root structures. Therefore, as Dr. Reski has testified, it is a fact that a person of ordinary skill in the art would have no expectation of success that, by attempting to combine the teachings of the Raskin Patent with one or more of the Houba-Hérin

[2] If one of ordinary skill in the art were to combine the references as suggested, they would further cultivate the transiently transformed protoplasts without selection markers until the protonema stage and then expect that the heterologous protein would be secreted into the culture medium. The protoplasts discussed in Houba-Hérin do not have cell walls. There is also no cell division in the short-term experiment. That means that no selection markers are used. The problem of selection of positively transformed cells does not arise in the Houba-Hérin article. If one were to further cultivate the transformants in Houba-Hérin, they would build cell walls and divide (into many parts) in order to produce a protonema. Then we would have reached the process step of Reutter et al. Because already many divisions would have take place, the constructs characterized in terms of a transient transformation in protoplasts would nearly have vanished, And because no selection marker is used, all the division products would become equal and one could no longer tell which are transformed and which are not. One of ordinary skill in the art would not accept this because it would **not** lead to a successful cultivation of a protonema tissue from the transiently transformed protoplasts to establish a continuous production of a heterologous protein.

article, the Reutter article, and the Nasu article, one would arrive at Applicants' claimed invention (Reski Declaration, ¶ 47).

For all of the above reasons, the Examiner has failed to demonstrate a suggestion, grounded in the art of record, for making the proposed combination of teachings and the Examiner has failed to demonstrate that the references would teach a reasonable expectation of success in arriving at the presently claimed invention even if the references could be combined.

III. CONCLUSION

Claims 1-6, 17 and 19-21 are now in compliance with 35 U.S.C. § 112 and are fully enabled by the disclosure as originally filed. Claims 1-6 and 17 pertain to a method for the production of heterologous proteinaceous substances in protonema tissue, and comply with the enablement requirement of 35 U.S.C. § 112, first paragraph, for the reasons described above. The Section 112, first paragraph, enablement rejection, however, is untenable for multiple reasons. First, the Examiner has failed to establish a prima facie case regarding lack of enablement because the Examiner has not adduced any evidence that bryophytes are biologically unpredictable, and/or poorly understood, organisms such as could even raise an issue regarding enablement. Second, the Examiner has failed to properly analyze the Wands factors, all eight of which weigh in favor of enablement, because the Examiner has considered each factor as a single, unrelated factor instead of considering all of the factors together. Third, the Examiner has put forth absolutely no evidence regarding the enablement rejection as applied to the present claims. And fourth, the process of transforming bryophytes is not presently recited as a step of the claimed invention. Instead, the presently claimed method, in accordance with the present invention, cultures transformed protonema tissue, but the process by which such tissue is created is not a limitation of the present claims.

The rejection of claims 1-6, 17 and 19 under 35 U.S.C. § 103(a) is untenable and must be withdrawn because the scope and content of the teachings of the asserted references is insufficient to sustain the rejection. Specifically, none of the Houba-Hérin article, the Reutter article, the Raskin Patent, nor the Nasu article teach, or even suggest, the steps of (a) culturing... protonema tissue transformed with a construct encoding a signal peptide operably linked to a protein ...; and (b) obtaining secreted heterologous proteinaceous substances from the culture

medium without disrupting producing tissues or cells as recited in independent claims 1, 17 and 19 of the present application.

The Section 103 rejection is also untenable and should be withdrawn because the Examiner has failed to demonstrate a motivation to combine the references that is grounded in the prior art, and the Examiner has failed to show that a person of ordinary skill in the art would have a reasonable expectation of success of arriving at the claimed invention when combining the teachings of the references.

For all of the above reasons, claims 1-6, 17 and 19-21 are in condition for allowance, and a prompt notice of allowance is earnestly solicited.

Questions are welcomed by the below-signed attorney for applicants.

Respectfully submitted,
GRIFFIN & SZIPL, P.C.

Joerg-Uwe Szipl
Registration No. 31,799

GRIFFIN & SZIPL, P.C.
Suite PH-1
2300 Ninth Street, South
Arlington, VA 22204

Telephone: (703) 979-5700
Facsimile: (703) 979-7429
Email: GandS@szipl.com
Customer No.: 24203